understanding of disease progression. What were once thought to be merely random events in cancer evolution are now being recognized as important mutational signatures in the biography of a human tumor.

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It Takes KASH to Hitch to the SUN

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LINC complexes are structures embedded within the nuclear envelope that mechanically couple the nucleus and cytoskeleton. They consist of SUN domain proteins of the inner nuclear membrane associated with KASH domain proteins in the outer nuclear membrane. Atomic resolution structures of SUN-KASH pairs now provide new insight in to the mechanisms of LINC complex assembly.

Observations stretching back more than two decades have suggested that nuclei and nuclear components are mechanically coupled to the cytoskeleton. In multicellular organisms, this mechanical coupling may extend beyond the plasma membrane to the extracellular matrix and adjacent cells. More recently, studies on a variety of human diseases associated with defects in nuclear envelope (NE) proteins have revealed that, not only can the cytoskeleton affect nuclear organization, but changes in nuclear architecture may have a reciprocal affect on cytoskeletal function (Burke and Roux, 2009). In this issue, Sosa et al. (2012) provide an atomic resolution description of key interactions at the NE that link nuclear and cytoplasmic components.

The most prominent features of the NE are inner and outer nuclear membranes (INM and ONM) separated by a gap, or perinuclear space (PNS), of about 40–50 nm. The two membranes are spanned by nuclear pore complexes at annular junctions. The ONM also displays connections to the endoplasmic reticulum (ER). In this way, the ER, ONM, and INM represent separate domains within a single continuous membrane system, with the PNS forming an extension of the ER lumen. Whereas the INM contains a unique array of membrane proteins, the composition of the ONM closely resembles that of the ER. Nevertheless, the ONM is enriched in several integral membrane proteins that function as adapters for a variety of cytoskeletal components, including motor proteins. In vertebrate cells, these ONM proteins are represented, in part, by members of the Nesprin or Syne family.

The common feature of all known ONM-specific proteins, including Nesprins, is a C-terminal KASH (*K*larsicht, *A*nc1, *S*yne *h*omology) domain of 50–60 amino acid residues (Burke and Roux, 2009). The KASH domain consists of a single-membrane spanning helix followed by a sequence of about 30 residues that extends into the PNS. Localization of KASH domain proteins to the ONM is dependent upon interaction with integral proteins of the INM that belong to the SUN (Sad1p, Unc84) domain family. In mammalian cells, there are two widely expressed SUN domain proteins, Sun1 and Sun2. The SUN domain itself is a highly conserved ~200 amino acid C-terminal sequence that resides within the PNS at the end of a helical "stalk" and can interact directly with KASH domains of ONM proteins. In this way, proteins such as Sun1 and Sun2 function as transluminal tethers for Nesprin proteins in the ONM.

The nucleoplasmic domains of SUN proteins are associated with a variety of nuclear components, including the nuclear lamina, an important structural feature of the NE that is closely associated with both the INM and underlying

chromatin. Together, pairs of INM SUN proteins and ONM KASH proteins form so-called linker of nucleoskeleton and cytoskeleton (LINC) complexes that physically couple elements of the cytoskeleton to nuclear structures (Figure 1).

SUN proteins are known to form oligomers. Consistent with this, recent crystallographic studies on human Sun2 have established that its SUN domain assembles into a clover-like trimeric structure (Zhou et al., 2012). This trimerization is mediated by sequences within the lumenal "stalk," which forms a coiled-coil triple helix. The SUN domain itself is folded in to a β sandwich. Each SUN domain within the trimer forms extensive contacts with its two neighbors. As revealed by Sosa et al. (2012), an important aspect of these interactions is mediated by a 20 amino acid loop, or "KASH lid," that forms a protruding antiparallel β sheet that overlaps the adjacent subunit. From an evolutionary perspective, the SUN domain most closely resembles certain fucose-binding lectins, suggesting that these proteins share a common ancestor.

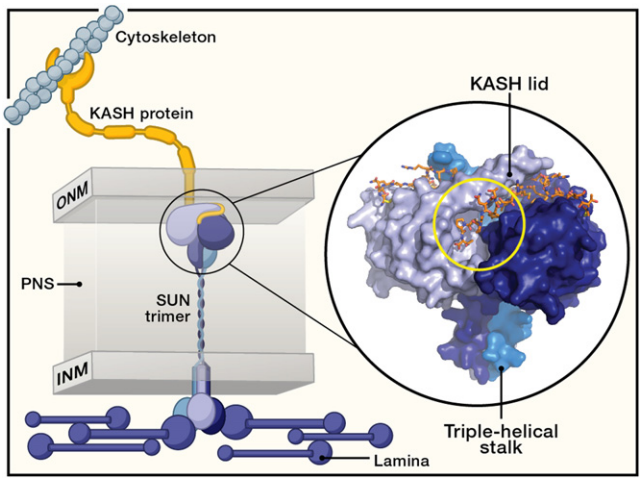

Figure 1. LINC Complex Organization

A trimer of SUN domain proteins from the inner nuclear membrane (INM) functions as a tether for a KASH domain protein of the outer nuclear membrane (ONM). The SUN and KASH domains interact directly within the perinuclear space (PNS). The enlarged image on the right (reproduced from Figure 2E of Sosa et al. [2012]) shows the KASH domain (orange) nestling between the "KASH lid" and a β sandwich of neighboring SUN monomers. The yellow circle highlights the KASH C terminus, which is accommodated within a deep pocket of one SUN monomer. These SUN-KASH pairs represent links in a molecular chain that mechanically couples the cytoskeleton to nuclear components, including constituents of the nuclear lamina. For simplicity, the KASH domain protein is depicted as a monomer. In reality, these may exist as oligomers. Furthermore, each SUN trimer may accommodate up to three KASH domains, raising the possibility that LINC complexes might form extended arrays in the NE.

Key to our understanding of LINC complex assembly is the nature of the SUN-KASH interaction. Cocrystallization studies by Sosa et al. now demonstrate that KASH domain binding involves two adjacent SUN domains. Deletion of the helical stalk, which prevents SUN domain trimerization, abolishes KASH binding. The KASH domain itself adopts an extended conformation snaking along a groove formed, in part, at the interface between the KASH lid of one SUN domain and the upper region of the β sandwich of its neighbor. The C terminus of each KASH domain, which typically features a cluster of two to three prolines immediately prior to the C-terminal amino acid, is accommodated in a deep pocket formed within the surface of a single SUN domain. This region of the KASH domain is critical for binding because extension of the C terminus by only a single amino acid eliminates SUN-KASH interactions. This result suggests that targeting the pocket with peptide-mimetic drugs could block LINC complex assembly.

LINC complexes have been implicated in a number of essential cellular and developmental processes, including nuclear anchoring, nuclear migration, and meiotic chromosome dynamics (Burke and Roux, 2009; Hiraoka and Dernburg, 2009). For each of these, stability of the SUN-KASH interactions is paramount. It is clear from the results described by Sosa et al. that SUN and KASH domains display extensive mutual contacts. Moreover, they demonstrate that a highly conserved cysteine residue within the KASH domain is ideally positioned to form an interchain disulphide bond with a corresponding cysteine within the SUN domain, providing additional LINC complex stability.

Though this work provides us with our first high-resolution view of LINC complex assembly, Sosa et al. present new issues to ponder. The first of these concerns the oligomeric status of ONM Nesprins and other KASH domain proteins. Although there is compelling evidence that these do indeed self-associate (Mislow et al., 2002; Ketema et al., 2007), whether they form dimers, trimers, or other higher-order structures is unknown. The nature and plasticity of such Nesprin oligomers would dictate whether LINC complexes might form extended and varied—depending on the combination of Nesprin proteins assembled on the SUN protein trimers—arrays in the plane of the nuclear membranes. Hints that these arrays could form come from observations of clustered LINC complexes in meiotic cells associated with NE attachment sites for telomeres (Ding et al., 2007) and in migrating fibroblasts, where they are aligned with actin filaments (Luxton et al., 2010).

A second issue that is raised by these studies concerns LINC complex remodeling. This is particularly relevant during mitosis, when the NE breaks down and nuclear membrane components are dispersed within the bulk ER. It seems unlikely that SUN-KASH interactions could be maintained during this period. Consequently, there must be mechanisms to reversibly break SUN-KASH interactions. The ER chaperone and AAA ATPase, Torsin A, has been suggested for exactly this role (Vander Heyden et al., 2009). The existence of an interchain disulphide bond that stabilizes SUN-KASH interactions also suggests that a member of the protein disulphide isomerase family might have a role in LINC complex dynamics.

Defects in NE proteins are associated with a variety of human diseases. In particular, certain mutations in A-type lamins, intermediate filament family proteins that are important constituents of the nuclear lamina, result in striated muscle diseases such Emery-Dreifuss muscular dystrophy (EDMD) and dilated cardiomyopathy. Consistent with these

findings, mice lacking a functional A-type lamin gene (*Lmna*) develop an EDMD-like syndrome soon after birth and die within 6 weeks of cardiovascular disease. A recent publication by Chen et al. (2012) reports the remarkable finding that down-regulation of Sun1 expression in *Lmna*-deficient mice significantly extended their life span along with amelioration of cardiac pathology. Thus, LINC complexes could represent viable therapeutic targets for the treatment of certain types of striated muscle disease and perhaps other laminopathies. The insights that Sosa et al. now provide into the molecular details of LINC complex assembly suggest strategies for the design of drugs that could interfere with SUN-KASH interactions. This convergence of structural biology and mouse genetics may provide the foundation for novel interventions in muscular dystrophy and heart disease.

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Ironing Out Cell Death Mechanisms

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Historically, key cellular regulators of diverse physiological processes have been uncovered by studying the mechanisms by which chemical entities produce interesting biological phenotypes. In this issue, Dixon et al. interrogate compounds that selectively kill oncogene-expressing cells, providing support for the existence of an iron-requiring, regulated form of cell death, ferroptosis.

Various forms of regulated cell death have been identified, including apoptosis, necroptosis, paraptosis, parthanoptosis, pyroptosis, and autophagic cell death. In this issue, Stockwell and colleagues propose the existence of ferroptosis, an iron-dependent form of cell death (Dixon et al., 2012). Ferroptosis requires iron-dependent production of reactive oxygen species (ROS), involves nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidases and lipid peroxidation, and is associated morphologically with the presence of shrunken, electron-dense mitochondria (Figure 1). Distinguishing it from many other forms of regulated cell death, ferroptosis does not require caspases (mediators of apoptosis and pyroptosis), ATP depletion or mitochondrial ROS generation (mediators of necroptosis), Bax/Bak (essential mediators of mitochondrial outer membrane permeabilization, MOMP), or elevations in intracellular Ca^{2+}.

Ferroptosis was uncovered while seeking an understanding of the mechanism underlying the activity of erastin, a small molecule that selectively kills cells expressing oncogenic mutants of RAS (Dolma et al., 2003). This quinazoline compound binds certain voltage-dependent anion channels (VDACs) on the mitochondrial outer membrane. Moreover, short hairpin RNA (shRNA) reagents targeting VDAC2 and VDAC3 rescue oncogenic RAS-expressing tumor cells from erastin (Yagoda et al., 2007; Yang and Stockwell, 2008). In the current paper, Dixon et al. (2012) show that erastin binds the subunit of cell surface amino acid transporters that import cystine, which then presumably causes reductions in glutathione, sensitizing cells to ROS. Evidence that cystine transport is critical to the mechanism of erastin includes data from experiments that either pharmacologically bypass erastin-induced cysteine transport block or that block cysteine transport with other compounds such as sulfasalazine and glutamate. The authors also sensitized tumor cells to erastin by shRNA-mediated knockdown of a subunit (SLCA11) of amino acid transporters